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Fermentative hydrogen production – An alternative clean energy source

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ABSTRACT

Hydrogen generation from wastewater is one of the promising approaches through biological route. So, exploitation of wastewater as substrate for hydrogen production with concurrent wastewater treatment is an attractive and effective way of tapping clean energy from renewable resources in a sustainable approach. In this direction, considerable interest is observed on various biological routes of hydrogen production using bio-photolysis, photo fermentation and heterotrophic dark fermentation process or by a combination of these processes. Therefore, in this communication, utilizing industrial wastewater as primary substrate for dark fermentation process is reviewed and different parametric aspects associated with this sustainable approach for better energy production is discussed. The industrial wastewaters that could be the source for bio hydrogen generation, such as rice slurry wastewater, food and domestic wastewaters, citric acid wastewater and paper mill wastewater, are also discussed in this article.

Contents

1. Introduction

The recent oil crisis and the consequent price rises have spawned considerable interest in the exploration of renewable energy sources. High concentrations of GHGs, resulting into rise of the average surface temperatures as well as adverse effects on weather patterns, human and animal life, are also responsible for the search of renewable energy sources [1,2]. Climate change, along with the rapid depletion of oil and gas reserves, has prompted many to search for eco-friendly energy alternatives, ideally one from the renewable sources. Hydrogen has been identified as a clean energy carrier and is found to be one potential alternative to fossil fuel

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Table 1
Summary of characteristics of biological hydrogen production processes.

Bio-hydrogen system	H_2 production rate (mmol H_2)	Required inputs and broad classification of microorganism used	By-products	General reactions and advantages
Direct biophotolysis	0.07	Light energy, H_2O, CO_2, trace minerals • Micro-algae	O_2	$2H_2O + light = 2H_2 + O_2$ It can directly produce H_2 directly from water and sunlight.
Indirect biophotolysis	0.355	Light energy, H_2O, CO_2, trace minerals • Micro-algae, cyanobacteria	O_2	$12H_2O + light = 12H_2 + O_2$ It has the ability to fix $N_2$from atmosphere.
Photo-fermentation	153.0	Light energy, H_2O, glucose • Purple-bacteria, microalgae	CO_2	$CH_3COOH + 2H_2O + light = 4H_2 + 2CO_2$ A wide spectral light energy can be used by these bacteria.
Dark fermentation (i) Mesophilic (ii) Thermophilic (iii) Extreme thermophilic	121.0 8.2 8.4	H_2O, carbohydrates, heat (ii and iii only) • Fermentative bacteria	CO_2, CH_4, CO, H_2S, acetate or other end products	$C_6H_{12}O_6 + 6H_2O = 12H_2 + 6CO_2$ It can produce H_2 all day long without light. A variety of carbon sources can be used as a substrate. It produces valuable metabolites such as butyric, lactic and acetic acid as a by-products. There is no O_2 limitation problem.
Hybrid reactor system		Light energy, H_2O, carbohydrates • Fermentative bacteria followed by anoxygenic phototrophic bacteria	CO_2	Stage-I: $C_6H_{12}O_6 + 2H_2O = 4H_2 + 2CH_3COOH + 2CO_2$ Stage-II: $CH_3COOH + 2H_2O + light = 4H_2 + 2CO_2$ Two stage fermentation can improve the overall yield of hydrogen

energy and has drawn a worldwide attention as a future energy source.

Current hydrogen generation methods can be grouped into two broad categories; conventional and alternative. Conventional hydrogen production methods mainly involve fossil fuel reforming, a process where, again, large amounts of CO_2 are generated. Alternative methods of hydrogen generation include electrolysis of water, biophotolysis and biological production from waste organic material. Among all the novel processes, biological hydrogen production has two main advantages over the conventional methods; it generates less GHGs and couples the metabolic activity of hydrogen-emitting micro-organisms with the simultaneous disposal of human-derived wastes rich in organics. Waste is generated everywhere in the form of solid, liquid or gas. The liquid waste is the wastewater, which is supplied to the communities and is drained out, when it is either unused or polluted by different sources. Liquid effluents from the residences and industries get mixed with the groundwater and storm water.

For over two decades, environmental engineers have successfully developed and commercialized anaerobic treatment technology for the treatment of wastewater [3]. In this process, organic pollutants and wastes are finally converted into methane through a series of chain reactions by distinct groups of anaerobic microorganisms. Complex organics are first hydrolyzed and fermented into fatty acids, which are then further converted into acetate and hydrogen, both of which are lastly converted into methane. As compared to the aerobic wastewater treatment process, the methanogenic process offers several intrinsic advantages: (a) saving the energy which is otherwise needed for aeration, (b) lowering of the sludge yield, and (c) producing a readily useable fuel-methane. Over 2000 full scale methanogenic wastewater treatment systems have been installed world over [3,4], where wastewater as primary fermentative substrate facilitates the wastewater treatment apart from the hydrogen production, which is considered to be economically viable. Industrial wastewaters contain high level of easily degradable organic material, which results in a net positive energy or economic balance, even when heating of the liquid is required. Industrial wastewaters as fermentative substrate for hydrogen production easily address most of the criteria for substrate viz., availability, cost and biodegradability.

Recently a new anaerobic process has been developed to convert organic pollutants into hydrogen, instead of methane. Hydrogen is favored over methane for two reasons. Firstly, hydrogen has a wider range of industrial applications as compared to methane. It can be used for the synthesis of ammonia, alcohols and aldehydes, as well as for the hydrogenation of edible oil, petroleum, coal and shale oil [5]. On the other hand, methane is mostly used as fuel. Secondly, hydrogen is an ideal fuel, producing only water upon combustion. It can also be used directly in the internal combustion engines and in the production of electricity through fuel cells [5–8]. Many energy experts believe that hydrogen can replace the fossil fuels as the next generation of energy [9–16].

This paper aims to present basic knowledge for the processes involved in the use of industrial wastewater for energy generation with suitable approach, and also to review the parameters that affect wastewater composition. This review gives useful references mainly concerning with the use of industrial wastewaters for the bio-hydrogen production by dark fermentation process and parametric aspects associated with the process.

2. Type of BHP methods

Biohydrogen is an important renewable energy carrier and its potential as next generation fuel needs to be explored [17–21]. Hydrogen metabolism is primarily the domain of bacteria and micro-algae. Within these groups, it involves many microbial species, including significantly different taxonomic and physiological types, various enzymes and metabolic pathways. Many approaches to the biological production of hydrogen exist, with varying production rates, input requirements and by-products.

Several approaches have been tried to take advantage of the bacterial hydrogen producing metabolism. Currently, the main processes for biohydrogen production include: (1) biophotolysis of water using algae/cyanobacteria, (2) photodecomposition of organic compounds using photosynthetic bacteria, (3) fermentative hydrogen evolution using anaerobic bacteria and (4) hybrid systems combining the fermentative and photosynthetic approaches either directly or in a series-type configuration. A novel technology, which focuses on combining the biological hydrogen production and electricity generation inside a microbial-fuel cell (MFC), is the future application.

Table 1 summarizes the various biological hydrogen production processes with general technical information involved therein, broad classification of microorganisms used with their relative advantages.

Among all these processes, photo and dark fermentation are important part of biological hydrogen production technologies. Between these two processes, dark fermentation seems to possess more advantages, such as more moderate reaction conditions, higher production rate, and proper liquid end products that are easy to use for the downstream processes. Accordingly, dark fermentation producing hydrogen has been more attractive in recent years. Dark fermentation has been suggested as more practical than the other processes as it does not require external energy to drive the process or large surface area to capture the necessary light. It can take advantage of existing reactor technologies to utilize organic wastes as feedstock [12,22,23].

In nature, it may be produced biologically by autotrophs as well as heterotrophs [11,24]. Autotrophs, such as algae, use carbon dioxide as carbon source, whereas heterotrophs use organic matter as carbon source. From an environmental engineering point of view, heterotrophs are of more concern because they can be used to degrade organic pollutants and thus clean up the environment. Heterotrophs produce hydrogen by either phototrophic or non-phototrophic (often called "dark") fermentation of organic matter; depending on whether light is the energy source. Most of the studies on heterotrophic hydrogen production since 1960s [25–28] have been related to non-phototrophic fermentation. The scarcity of information related to phototrophic fermentation is due to two reasons: (a) it is difficult to control light penetration and its uniform distribution, and (b) the process is likely not cost-effective unless the free sunlight can be used as the light source as discussed in Table 1. On the other hand, non-phototrophic fermentative hydrogen production mostly depends upon the pure cultures, but environmental engineers are mostly interested in using mixed cultures for wastewater/waste treatment for practical reasons. A mixed culture system would be cheaper to operate, easier to control and having a broader choice of feedstock [29]. This key process of biological hydrogen production depends upon process and performance parameters.

2.1. Process/operational parameters: includes pH, temperature, hydraulic retention time (HRT), reactor type, substrate type, cultures and nutrient availability.

2.2. Performance parameters: include hydrogen content in biogas (%), conversion efficiency (%), yield (mL-H_2/g-hexose), maximum volumetric production rate (L-H_2/L/d), and maximum specific rate (L-H_2/g-VSS/d).

In this paper, only articles related to dark fermentation for hydrogen production by pure/mixed cultures are reviewed.

3. Various operational parameters

Important process and performance parameters are discussed in this section. Over 160 publications related to fermentative hydrogen production from industrial wastewater by pure/mixed cultures are compiled and analyzed. In most of the reported cases, actual wastewater and synthetic wastewater are used as substrate for hydrogen production.

3.1. Substrate type: industrial wastewater (actual and synthetic)

Industrial wastewater is heterogeneous, both in composition and volume. Industries generate wastewater that contains a mixture of different pollutants, which often suffer from low biodegradability and are recalcitrant to biological treatment. Owing to a great variability of chemical structure and properties of compounds present in industrial wastewater and the risk of toxicity, conventional biological processes, even though commonly used due to their low costs, are seldom efficient. There is an urgent need of developing innovative treatment technologies capable of degrading toxic or refractory pollutants present in the wastewater. The data regarding the wastewater generation from the Indian cities is presented in the following Table 2 [30]. The total quantity of wastewater generated is estimated to be 15,392 million liters per day (Mld) where as only 10,170 Mld, which is about 66% collected through sewerage system, is treated conventionally. The treated sewage results in large quantity of sludge which can be converted to biogas using dark fermentation/anaerobic digestion.

Table 2
Wastewater generation in India [30].

Name of the zone	City classification	Wastewater generated (Mld)	Wastewater collected (Mld)
South	Very big	669.53	
	Big	58.22	
	Medium	640.42	
	Small	1532	
		2911	1812
North	Very big	1935	
	Big	394	
	Medium	948.26	
	Small	2250	
		5578	3932
Western	Very big	978	
	Big	437	
	Medium	780.525	
	Small	1269	
		3469	2275
Eastern	Very big	55	
	Big	297	
	Medium	631	
	Small	2461	
		3434	2151

H_2 production potential has been found to be dependent on the nature of wastewater, wastewater composition/biodegradability, reactor configuration, mode of reactor operation, inlet pH and applied substrate loading rate. Exploitation of inexhaustible quantities of wastewater and biomass as primary substrate for H_2 production with simultaneous treatment can be considered as viable and green alternative. Utilization of wastewater as fermentable substrate and feasibility to operate with mixed culture at ambient temperature and pressure make this process less energy intensive and more environmental friendly.

Extensive research has been carried out for hydrogen production using various waste materials like wastewater from industrial process such as rice slurry wastewater [31], food and domestic wastewaters [32–34], citric acid wastewater [35]. Use of industrial wastewater as substrate facilitates both treatment and renewable extraction of clean gas simultaneously.

3.1.1. Food processing industries

Effluents from the Food and Beverage industry contain the highest concentration of organic compounds in the form of carbohydrates. These effluents mainly generated from agricultural processes (such as animal wastes and agricultural residues) and food industries (such as dairy processing and vinery wastewaters) [31,36,33,37] are preferred for economic reasons.

The most important industries are Food and Beverage industries [38], Slaughter houses and meat-processing, Dairy, Fish-processing, Starch-processing, Sugar, Edible oil, Beverages and distilleries, Fruit and vegetable processing, and Coffee processing.

California is the largest dairy product producing state in the United States and thus, it also produces the greatest amount of whey as a by product. So, Yang et al. [34] developed a stable

anaerobic fermentation process for continuous H_2 production by using batch and continuous anaerobic fermentation of cheese whey wastewater by mixed microbial cultures. Similarly, India also has an opportunity for developing an innovative technology for utilization of this type of by-product as a valuable resource like hydrogen. From Indian point of view, this process technology is important from both an economical and environmental angle as this country has got a good number of dairies and also requires excess energy for its growing economy.

Moreover, organic content rich wastewaters such as sugary wastewater [39] and starch manufacturing waste [40,41], probiotic wastewater [42] have been used as substrates for hydrogen production with the advantages of reducing cost of substrate for hydrogen fermentation and also disposal cost of wastes. India is the second largest producer of sugarcane next to Brazil. Presently, about 4 million hectares of land is under sugarcane cultivation with an average yield of 70 tons per hectare. Sugar factory operations generate bagasse as a solid waste, and other process wastes include wastewater and pressmud. The major process wastewater streams from the sugar industry are milling, cooling water, boiler blow down, sulfur house, lime house and spray pond overflow. These wastewater have been used for anaerobic digestion and production of biohydrogen [43,44]. The total quantum of wastewater generated from 431 sugar factories will be up to 534,000 cum/day, which means 534,000 m^3 of biogas can be generated per day from the digestion of wastewater coming only from the sugar industry. The total biogas generation potential utilizing pressmud obtained from all the 431 sugar factories is estimated to be 2.32 mm^3/day [32].

3.1.2. Chemical industries

Chemical wastewater may act as primary carbon source in the metabolic reactions involving molecular H_2 generation leading to substrate degradation. Mohan et al. [45–47] have done a lot of work on the improvement in the rate of H_2 production from anaerobic treatment of chemical wastewater by successful application of bioaugmentation strategy. The augmented culture persisted in the system till the termination of the experiments. The survival and retention of the augmented inoculum and its positive effect on process enhancement has been attributed to the adopted reactor configuration and operating conditions. Scanning electron microscope (SEM) images have documented the selective enrichment of morphologically similar group of bacteria capable of producing H_2 under acidophilic conditions in anaerobic microenvironment.

3.1.3. Paper mill industries

Paper Industry is more than a century old and has grown admirably by adapting to a wide range of locally available cellulosic fiber resources. The manufacturing of paper generates significant quantities of wastewater; as high as 60 m^3/ton of paper produced. The raw wastewaters coming from paper and board mills can be potentially very polluting and is found to have the COD value as high as 11,000 mg/L [48]. There are about 51 agro-based mini and medium capacity mills with a total capacity of 2921 tpd (tons per day). The total daily bioenergy generation potential from the anaerobic treatment of black liquor from all these units will be 412,278 m^3/day [32].

3.1.4. Distillery industries

Disposal of distillery waste is a great challenge for many distilleries. Distillery waste is acidic in nature (pH 3.5–4.5), dark brown in color, has high COD (70–80 g/L) and BOD (40–50 g/L). Distillery waste also contains salts to the extent of 25–30 g/L. About 10–12 L of wastewater (called as distillery waste or spent wash) is generated for every liter of alcohol distilled. The major process wastewater stream coming from the distillation stages is the 'spent wash' and is regarded as a high strength waste having large potential for generation of bioenergy using anaerobic digestion [49–53].

Ethanol production is an important industry in India. Maharashtra is the leading state with 62 distilleries out of the total 300 molasses based alcohol distilleries in India [54]. H_2 production from distillery effluent was investigated at lab-scale and pilot-scale both. The seasonal variations on the molasses quality, particularly sugar content, sulfur content, and their influences on H_2 production using individual and/or co-culture of *Citrobacter freundii* and/or *Rhodopseudomonas palustris* P2 were investigated [55]. Vatsala and Manimaran [56] have also reported H_2 production from distillery effluent at 10 m^3 volume and the produced H_2 was subsequently tested for fuel cell application.

Vatsala et al. [57] also demonstrated the pilot scale study (100 m^3) on the utility of distillery effluent as an clean energy source using co-cultures of *C. freundii* 01, *Enterobacter aerogenes* E10 and *R. palustris* P2, which accounted for 21.38 kg of H_2 in 40 h. Authors have co-related the output results in terms of energy, i.e. 3.045 Gj, based on the upper combustion value of 7.18 Mj/Nm^3. In parallel, a high level of reduction in BOD and COD was also achieved in 40 h.

From pollution control and resource recovery point of view, it would be ideal if one can convert polluted wastewater into hydrogen energy. However, in this extensive research review article, few studies have been conducted for hydrogen production from actual wastewater, and some of the researches carried out by after the preparation of synthetic wastewater as a substrate [58–62].

3.2. Hydrogen producing bacteria

Fermentative conversion of substrate to H_2 is generally manifested by diverse group of specific bacteria by a complex series of biochemical/metabolic reactions and requires considerable optimization prior to scaling up. Low substrate conversion efficiency to H_2 is one of the significant problems encountered in the fermentative process. Most of the organic fraction remains as soluble fermentation products. Hydrogen production is a specific mechanism to dispose off excess electrons through the activity of hydrogenase enzyme in bacteria. Bacteria possessing such capability include strict anaerobes (Clostridia, Methylotrophs, Rumen bacteria, Methanogenic bacteria, Archaea), facultative anaerobes (*Escherichia coli*, *Enterobacter*, *Citrobacter*), and even aerobes (*Alcaligenes*, *Bacillus*) [63–66]. Among the hydrogen-producing bacteria, *Clostridium sp*. and *Enterobacter* are the most widely studied bacterial strains (Table 3).

Apart from pure cultures, various mixed micro-flora and co-cultures have also been explored for hydrogen production from carbohydrates [11,12,67,68]. Nevertheless, the quest for ideal microbe(s) for microbial H_2 production has been the major thrust for the researchers to screen various sources.

3.3. Nutrient availability

Like all fermentation processes, hydrogen production requires nutrients for bacterial metabolism, growth and activity. The nutrients include nitrogen (N), phosphate (P) and some trace elements. On the other hand, hydrogen production may also be inhibited by chemicals and the presence of other bacteria. The summary of the effects of nutrients and inhibitors are given below.

3.3.1. Nitrogen

Nitrogen is one of the most essential nutrients needed for growth. Liu and Shen [86] investigated the effect of N concentration, using NH_4HCO_3 as the N source, on the batch production of hydrogen from starch. A total of seven N concentrations were

Table 3
Various hydrogen producing microbial strains reported and their H_2 yield.

Organism	Substrate	Process	Maximum yield of H_2 (mol H_2/mol substrate)	References
Enterobacter aerogenes HU-101 (mutantAY-2)	Glucose	Batch (blocking metabolites formation)	1.17	[69]
Enterobacter aerogens	Molasses	Ar sparging, batch	1.58	[70]
Enterobacter aerogens	Molasses	Batch	0.52	[70]
Clostridium butyricum	Glucose	N2 sparging continuous	1.4–2.3	[71]
Enterobacter cloacae IIT BT 08	Glucose	Continuous (immobilized bioreactor)	2.3	[72]
Citrobacter sp. Y19	Glucose	Batch Ar sparging	2.49	[73]
Rhodopseudomonas palustris P4	Glucose	Batch, with intermittent purging of Ar	2.76	[74]
Clostridium butyricum EB6	POME	Batch	3.2 (L/L med)	[75]
Clostridium butyricum ATCC19398	Glucose	Batch	1.8	[76]
Clostridium acetobutyricum M121	Glucose	Batch	2.29	[76]
Clostridium tyrobutyricum FYa102	Glucose	Batch	1.47	[76]
Clostridium beijerinckii L9	Glucose	Batch	2.81	[76]
C. thermolacticum	Lactose	Continuous	3.0	[77]
Clostridium thermocellum 27405	Delignified wood fiber	Batch	1.6	[78]
Klebsiella oxytocoa HP1	Glucose	Batch	1.0	[79]
T. thermosaccharolyticum PSU-2	Sucrose	Batch	2.53	[80]
T. saccharolyticum JW/SL-YS485	Xylose	Batch	0.88	[81]
Caldicellulosiruptor	Sucrose	Batch	5.9	[82]
Mixed culture (predominantly Clostridium sp.)	Glucose	N2 sparging, continuous HRT: 8.5 h	1.43	[83]
Mixed microflora	Wheat starch co-product	N2 sparging continuous	1.9	[84]
Mixed microflora	0.75% soluble starch	Chemostat HRT: 17 h	2.14	[62]
Mixed microflora	Sewage-sludge	Anaerobic and acidogenic digestion	1.7	[85]

studied, varying from 0.1 to 2.0 g-N/L, corresponding to C/N ratios from 67 to 3.3. Results showed that the maximum hydrogen yield (175 mL-H_2/g-hexose) and specific hydrogen production rate (0.2 L-H_2/gVSS/d) at 1.0 g-N/L or C/N ratio of 6.7.

Morimoto et al. [87] compared the hydrogen yield from glucose (10 g/L) using yeast extract as N source at three concentrations, i.e. 0.2, 0.4 and 0.8 g-N/L assuming an average N content of 10% in yeast extract, corresponding to C/N ratios of 20, 10 and 5. Results showed that the highest hydrogen yield of 170 mL-H_2/g-hexose at 0.4 g-N/L concentration or at C/N ratio of 10. Lin and Lay [88] compared the hydrogen yields of sucrose at four concentrations with 0.9 g-N/L of nitrogen, corresponding to C/N ratios of 130, 98, 47 and 40. The highest hydrogen yield (327 mL-H_2/g-hexose) was obtained at C/N ratio of 47.

3.3.2. Phosphate

A few studies have shown that phosphate is needed in hydrogen production for its nutritional purpose as well as for buffering capacity [73,74,89].

3.3.3. Trace metals

Lin and Lay [90] studied the requirement of 11 trace metals in hydrogen production using the experimental design of Taguchi orthogonal arrays. They reported that magnesium, sodium, zinc and iron were of significance for hydrogen production; among the four, magnesium was the most significant one. Magnesium ion is an important co-factor that activates almost 10 enzymes including hexokinase, phosphofructokinase and phosphoglycerate kinase during glycolysis process [91]. Nearly all the other trace metal studies were focused on iron alone, probably because its presence is essential for hydrogenase [92,93]. Lee et al. [94] studied the effect of iron concentration on hydrogen production using seed sludge from digester as inoculums. The maximum hydrogen production rate was found to be 24 mL/g VSS/h at 4000 mg/L $FeCl_2$. Zhang and Shen [95] reported a antagonistic effect of temperature and iron concentration. Effect of iron for hydrogen production decreased with increasing reactor temperature, i.e. at 25 °C, hydrogen production (200 mg/L) peaked at $FeSO_4$ concentration of 800 mg/L and at 35 °C temperature. The researchers have suggested that when the ambient temperature is relatively lower, bacteria need more ferrous ion to activate the hydrogenase enzyme, so that it can oxidize reduced ferredoxin for better production of molecular hydrogen. Several other studies have also reported that iron-limiting conditions, not only lower the production of hydrogen as well as acid, but also increases the production of alcohols, such as ethanol and butanol [92,94].

3.3.4. Toxic heavy metals

Heavy metals, including cadmium, chromium, zinc, copper, nickel, and lead, may be present at significant concentrations in some industrial wastewater and municipal wastes. These metals are often found to be the leading cause of anaerobic reactor upset and failure. Fang [96] compared the effect of five heavy metals on the methanogenic granular sludge, and reported that toxicity was in the following order: zinc > nickel > copper > cadmium > chromium.

A similar order of metal induced inhibition of hydrogen production was also observed by other workers. Hsieh [97] reported that, for hydrogen production from sucrose, zinc (Ci_{50} 4.5 mg/L) was slightly more toxic than copper ($C_{I,50}$ 6.5 mg/L), which in turn was much more toxic than chromium ($C_{I,50}$ 60 mg/L). In two separate studies on hydrogen production from dairy waste water, copper ($C_{I,50}$ 65 mg/L) was reported to be more toxic than zinc ($C_{I,50}$ 120 mg/L) [98], and chromium ($C_{I,50}$ 72 mg/L,) more toxic than cadmium ($C_{I,50}$ 170 mg/L) [99].

3.4. Working parameters

System operating conditions significantly influence the overall performance of hydrogen production process in association with wastewater treatment.

3.4.1. pH

Bacteria respond to changes in internal and external pH by adjusting their activity and synthesis of proteins associated with many different processes, including proton translocation, amino acid degradation, adaptation to acidic or basic conditions and virulence. pH of the medium plays a crucial role in governing the metabolic pathways of organism, where activity of hydrogen producing bacteria is considered. Hydrogen production occurs at acidification stage of the metabolic process. System pH also influences efficiency of substrate metabolism, protein synthesis, synthesis of storage material and release of metabolic by-product. In most of the studies, butyrate and acetate were the two main products, while low pH seemed to favor butyrate production.

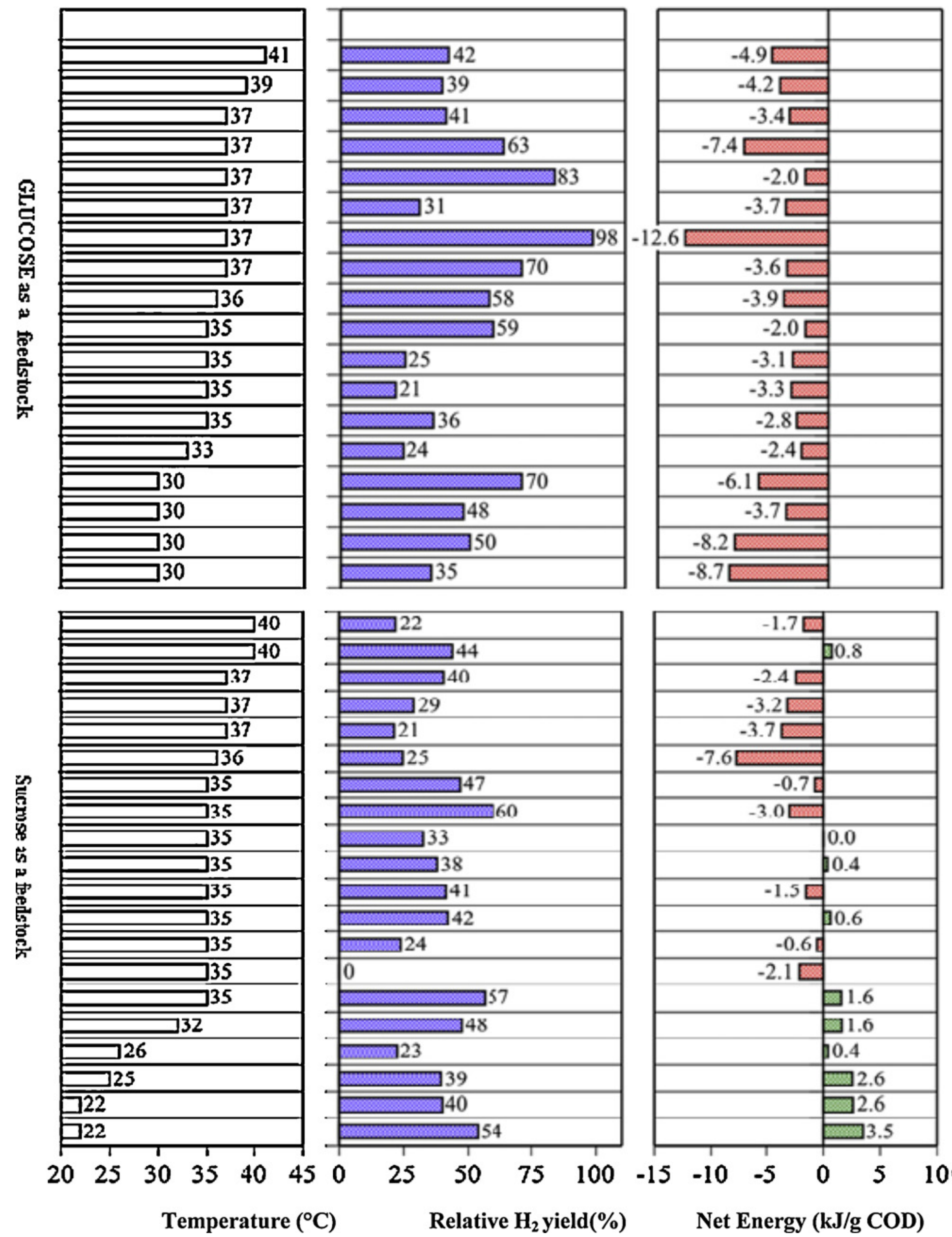

Fig. 1. Fermentation temperature, relative hydrogen yield, and net energy gain reported in the literature for glucose and sucrose [119].

Propionate production increased substantially at pH 7.0 and above. Fang and Liu [100] investigated the product profiles from pH 4.0 to 7.0. Butyrate was found to be the most predominant product (up to 45.6%) at pH 6.0 or below, whereas acetate became more predominant (up to 34.1%) at pH 6.5 or above.

Optimum pH range for hydrogen uptake bacteria is found to be between 6.0 and 7.5, while hydrogen producing bacteria function well below the pH 6.0 [101–104]. The pH range of 5.5–6.0 is ideal to avoid the process of methanogenesis, which is a key factor for effective generation of hydrogen. It is the only parameter which directly affects the hydrogenase activity [105]. Fang and Liu [100] investigated the effect of pH ranging between pH 4.0 and 7.0 (with increments of 0.5 pH), and concluded that the optimal pH was 5.5 with a yield of 286 mL-H_2/g-hexose and a specific production rate of 4.6 L-H_2/g-VSS/d.

For the degradation of simple substrates, the optimum initial pH for *Clostridia* is reported to be in the range of 4.5–7.0. Although high yields have been reported at a pH value as large as 9.0 [106–110].

Skonieczny and Yargeau [111] investigated a production of biological hydrogen from glucose by species *Clostridium beijerinckii* using synthetic wastewater solution. Effect of initial pH (range 5.7–6.5) and substrate loading (range 1–3 g COD/L) was studied. Results of the study demonstrated a strong trend of increasing hydrogen production rate with an increase in both substrate concentration and pH.

Production of acids is known for gradual decline in the buffering capacity of the system, which, in turn, results in a concomitant decline in the system pH. In experimental variations with respect to pH, accumulation of organic acids leads to process inhibition. Unless the pH is not maintained in the optimum range, there is cessation of hydrogen production along with marked shift in microbial population.

3.4.2. Temperature

The effect of temperature on hydrogen production was studied in several studies, most of which seemed to show that hydrogen

Table 4
Process and performance parameters for wastewaters with various bioreactors [160].

Feedstock	Reactor type	Seed sludge	pH	Temperature (°C)	HRT (h)	Hydrogen content (%)	Yield (L-H_2/kgCOD)
Sugar factory wastewater	CSTR	Compost	6.8	60	12	64	321
Wastewater containing sugar and ethyl alcohol	PBR	ADS	6.0–6.5	37	8	60	102
Noodle manufacturing wastewater	CSTR	ADS	5.2	35	18	–	187
Rice winery wastewater	PBR	AS	5.5	55	2	61	272
Food processing wastewater	Batch	Soil	4.0–6.4	23	–	60	100

Note. CSTR, continuous stirred tank reactor; PBR, packed-bed reactor; ADS, anaerobic digested sludge; AS, acclimated sludge.

yield increased with temperature [29,31,112,113]. All the hydrogen production studies listed in literature were conducted in three temperature ranges: ambient (15–30 °C), mesophilic (32–39 °C), and thermophilic (50–64 °C). However, due to the drastic differences in reactor, substrate, seed sludge and other process conditions, it is difficult to compare hydrogen yield at the three temperature ranges. The highest reported yields were 266 mL-H_2/g-hexose for 15–30 °C [114,115]; 333 mL-H_2/g-hexose [59] for 32–39 °C, and 327 mL-H_2/g-hexose for 50–64 °C [116].

However, an increase in temperature can also have detrimental effects on hydrogen production rates [117,118]. Lin et al. [117] have shown that even an increase of just 5 °C can have serious impact on hydrogen production rates by about 25%. These results of the study conducted by Perera et al. [119] after an extensive review of literature on fermentative hydrogen production suggested that net energy gain declined with increase in fermentation temperature and showed negative value once the fermentation temperature exceeded beyond 30 °C. The relevant literature and data related to glucose and sucrose as a fermentation substrate [58,61,83,109,120–142] are summarized in Fig. 1.

3.4.3. HRT

HRT is one of the most important control parameters affecting continuous production of hydrogen. HRT control can avoid the hydrogen utilization by hydrogen-consumers like methanogens [143]. However, the reported optimum HRTs for hydrogen production are rather inconsistent and varies from 8 h for sucrose [61] and 12 h for glucose [85] and 18 to 24 h for brewery waste water [144,145]. Besides, Zhang et al. [146] found increase in the hydrogen yield from 1.6 mol H_2/mol glucose to 1.9 mol H_2/mol glucose when the HRT was shortened from 8 h to 6 h.

3.5. Bioreactor type and operation

With respect to batch and continuous mode operation in hydrogen production, various types of reactors were employed [60,147–154], among which continuously stirred type bioreactors was mostly reported [52,61,145]. Batch reactors used for simple operation and efficient control. However, large scale operations would require continuous production processes for practical engineering reasons.

CSTR has been the most common mode of continuous hydrogen production. Complete mixing allows intimate contact between the substrate and biomass, as well as effective pH and temperature control. The common alternative to CSTR for the continuous hydrogen production is packed-bed reactor. In such a reactor, biomass are immobilized either in granules [103,155] or in biofilms [156], or entrapped in packed media [60]. The flow pattern within the packed-bed reactor is plug flow with little mixing. In most packed-bed reactors, wastewater enters at the bottom and exits from the top. This is commonly known as upflow packed-bed reactor. Those used for the downflow mode are also known as trickling biofilter reactors [156]. Wu et al. [157] studied the hydrogen production from a sucrose-rich wastewater by using immobilized biomass in alginate beads with acrylic latex and silicone inside- a three-phase fluidized-bed reactor. Results showed that the fluidized-bed reactor was more flexible and stable to operate with a yield of 182 mL-H_2/g-hexose and a production rate of 22.3 L-H_2/L/d. Yu et al. [31] used rice winery wastewater in an upflow sludge bed reactor, with a maximum yield of 291 mL-H_2/g-hexose and volumetric rate of 3.8 L/L/d. However, no granulation was observed in their study.

Membrane bioreactor has become a mature technology in aerobic wastewater treatment [158] and has recently been applied to the anaerobic process [159]. It relies on membrane to retain sludge in the mixed liquor by membrane separation, so that the reactor can be operated at high biomass concentrations with very low sludge yield. Table 4 depicts the performance of bioreactors with various industrial wastewaters.

4. Conclusion

Although the technical feasibility of fermentative hydrogen production from industrial wastewater has been demonstrated by various workers, the technology is still in its infancy. After reviewing related papers, it is obvious that further studies are required on a number of crucial areas for development of an efficient BHP technology.

It can be concluded that fermentation alone, even under the most ideal condition, can convert about 40% of the chemical energy of wastewater into hydrogen energy. The energy residues remaining as by-products in the form of acids and alcohols will require additional treatment processes for further energy recovery. A sustainable strategy has to be developed as a technology package, which not only includes just fermentation, but also improves the downstream process required for the full recovery of chemical energy from the wastewaters. Microbial fuel cells used for the direct production of electricity also present the possibility of being used for harnessing the energy trapped in the organic rich waste water. Although studies are now in full swing in theoretical and technical aspects, covering isolation of new bacterial strains, exploring the pathways efficient in the hydrogen production by using molecular biology and genetic engineering, reactor design and optimization, economically cost effective raw materials, etc. Considering all the potential advantages and challenges in the field of hydrogen production, particularly through dark fermentation, it is clear that microorganism based technique of hydrogen production is moving forward, albeit slowly, offers a very bright and promising future for achieving a sustainable and clean energy technology.

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